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SAR Studies of Indole-5-propanoic acid Derivatives to Develop Novel GPR40 Agonists

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KEYWORDS: GPR40 agonists, indole-5-propanoic acid, NFAT reporter assay, glucose-stimulated insulin secretion (GSIS).

ABSTRACT: G-protein coupled receptors 40 (GPR40) has been considered to be an attractive drug target for the treatment of type 2 diabetes because of its role in free fatty acids-mediated enhancement of glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells. A series of indole-5-propanoic acid compounds were synthesized and their GPR40 agonistic activities were evaluated by nuclear factor of activated T-cells (NFAT) reporter assay and GSIS assay in the MIN-6 insulinoma cells. Three compounds, 8h (EC_{50} = 58.6 nM), 8i (EC_{50} = 37.8 nM), and 8o (EC_{50} = 9.4 nM) were identified as potent GPR40 agonists with good GSIS effects.

Free fatty acids (FFAs) are an important source of energy for the body and play a role in signal transduction for insulin secretion and other cellular effects.[1, 2] G-protein coupled receptors 40 (GPR40) was identified as an orphan G-protein coupled receptors with an unknown function.[2] Recent studies revealed that GPR40 is abundantly expressed in pancreas and regulates insulin secretion from beta cells of the pancreas via long-chain FFAs.[3] Takeda has developed the GPR40 agonist TAK875 (Figure 1), which activates the glucagon-like peptide-1 (GLP-1) pathway as well as GPR40 and increases the secretion of insulin.[4] In 2011, a phase III clinical trial of TAK875 was initiated.[5, 6] The trial was the first attempt to develop a GPR40 agonist as a drug for type II diabetes mellitus. However, its development was discontinued due to potential hepatotoxicity.[7] Clinical developments of some other GPR40 agonists were also discontinued for undisclosed reasons. Recent studies suggested that the potential liver toxicity of TAK875 and other GPR40 agonists is associated with their analogous hydrophobic pharmacophore. Smaller and less lipophilic compounds have improved the drug-likeness properties.[8, 9] As shown in Figure 1, the common feature of most GPR40 agonists is a phenyl propanoic acid group and a benzyl oxy or benzyl amine group in the linker unit[10-15] which are very important for GPR40 agonistic effect.[16-18] The GPR40 agonist TUG-770 has a rigid alkyne group in the linker part,[10] which should provide a different ligand receptor binding interaction compared to flexible benzyl oxy or amino linker.[19]

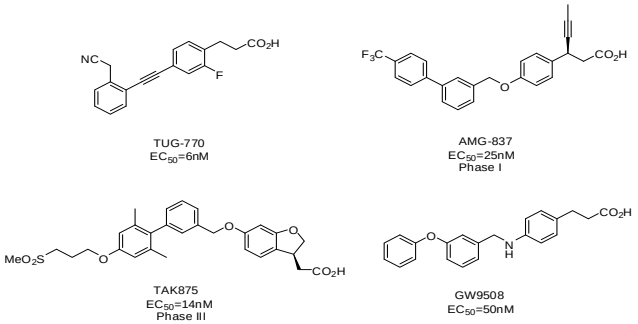


Figure 1. Reported GPR40 agonists

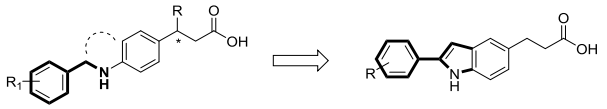
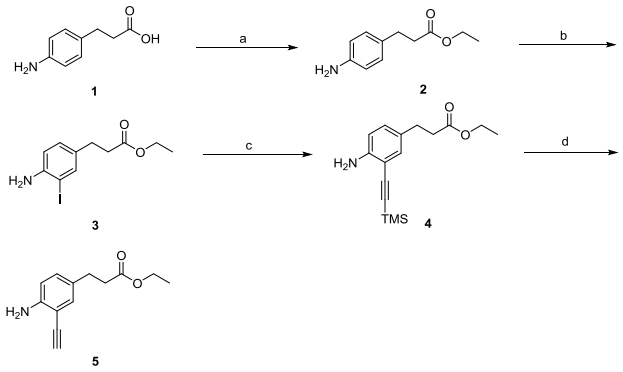


Figure 2. Design of indole propanoic acid

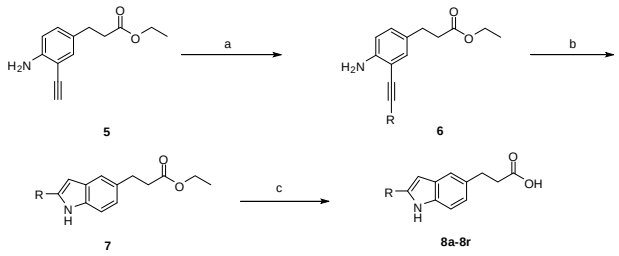
Another GPR40 agonist includes a derivative of benzofuran that was designed as a constrained planar derivative of benzyl oxy linker. Its agonistic activity is weaker than other derivatives having benzyl oxy or alkyne moieties in the linker part.[20] In a similar way to develop TAK875, various bicyclic acid moieties including 2-(1*H*-indol-1-yl)acetic acid or 3-(1*H*-indol-1-yl)acetic acid, are adopted in the acid tail part of GPR40 agonists with benzyl oxy linker. However, their activity are weaker than those with a phenyl propanoic acid tail (EC_{50} values ranging between 1 and 10 μM).[21] Based on these reports, we designed novel GPR40 agonists by employing an indole moiety in which a phenyl ring is fused to a pyrrole ring as a bioisostere of two-atom linker unit,[22] and a small-size aryl group to reduce the hydrophobicity (Figure 2). A series of 3-(2-aryl-1*H*-indol-5-yl) propanoic acid derivatives were prepared for the present structure-activity relationship study. We expected binding modes of these molecules would be similar to that of TAK875 to give an agonistic effect on GPR40,[23] although their indole linker unit is more rigid than the benzyloxy linker.

Scheme 1[a]



[a]**Reagents and Conditions**: (a) sulfuric acid, EtOH, reflux, 98%; (b) NIS, DMF, r.t., 90%; (c) TMS-acetylene, $PdCl_2(PPh_3)_2$, CuI, TEA, THF, 60°C, 73%; (d) TBAF(1M in THF), THF, r.t., 82%

Scheme 2[a]

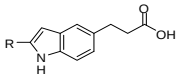


[a]**Reagents and Conditions**: (a) Aryl halide, $PdCl_2(PPh_3)_2$, CuI, TEA, DMF, 80°C; (b) $AuCl_3$, EtOH, 60°C; (c) NaOH, MeOH, 40°C

The synthesis of key intermediate **5** was achieved as shown in **Scheme 1**. The esterification of commercially available 3-(4-aminophenyl) propanoic acid **1** under sulfuric acid, provided compound **2**. To prepare 2-ethynylaniline **5**, the iodination of compound **2** was carried out first. The Sonogashira coupling reaction was conducted to replace iodo group with ethynyl group (compound **4)**, and then following TMS (trimethylsilyl ether) deprotection using TBAF (tetrabutylammonium fluoride) directly to afford compound **5**. Compound **5** is an important intermediate for the synthesis of indole propanoic acid derivatives, as presented in **Scheme 2**, because it can employ various aryl or alkyl group at the C2 position of indole ring. Among the various methods for the synthesis of indole, we used a method that cyclizes the acetylenic substituent onto the amino group.

Synthesis of indole propanoic acid derivative was accomplished as summarized in **Scheme 2**. Various phenyl groups were introduced to the key intermediate **5** by the Sonogashira reaction. In this reaction, the bulkiness of the ortho-substituent of aryl halide affected the yield, and the diyne compound was obtained as a main side product. For the synthesis of indole, gold chloride ($AuCl_3$) was used and the indole compound **7** was easily obtained under the mild reaction condition.[24] Finally, the resulting indole compounds were hydrolyzed to give the final products, **8a - 8r**.

Table 1. 2–Aryl substituted indole propanoic acid



Compd	R	EC_{50} (nM, hFFA1)[a]	ALog P[b]	PPAR γ at 10μM[c]
TAK875		14	5.06	
8a		N.A.	3.65	14%
8b		447	4.14	4%
8c		N.A.	4.14	8%
8d		N.A.	4.14	4%
8e		N.A.	3.63	0%
8f		N.A.	3.63	5%
8g		N.A.	3.63	5%
8h		54.6	4.31	1%
8i		37.8	4.31	101%[d]
8j		778	4.31	1%
8k		1800	4.39	56%

[a] Luciferase reporter assay, [b] AlogP [25] was predicted by schrödinger maestro, [c] % activity compared to 1μM rosiglitazone, [d] PPARγ EC_{50}=2.4μM, N.A. no activity (> 25 μM).

We conducted a nuclear factor of activated T-cells (NFAT)-luciferase reporter assay[26] in Chinese hamster ovary (CHO) cells to evaluate the GPR40 agonistic activities of the compounds in comparison with TAK875 respectively (Figure S1a in the Supporting Information). Our reporter assay system was validated using the reference molecule TAK875, giving the EC_{50} value of 14 nM (Table 1) which is similar to that from the

calcium influx assay.[11] In terms of lipophilicity, the calculated logP (ALogP) values for all of compounds, except **8m**, were less than that of TAK875 (5.06) indicating that hydrophobicity is reduced in indole propanoic acid derivatives as expected.

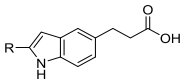
Since another antidiabetic target PPARɣ is also activated by fatty acids, it is plausible that GPR40 agonists may have PPARɣ agonistic activity.[27] Several indole-5-acetic acid and α- alkoxy-indole-5-propionic acid derivatives, which are structurally similar to our compounds, were also reported as PPAR agonists.[28, 29] Thus, we examined the PPARɣ transactivation activity of our compounds at 10 μM (Tables, 1 and 2) and most of them did not show significant PPARɣ agonist activity, except **8i**. The EC_{50} values for PPARɣ were determined for **8i**, **8k**, and **8r,** and they were 2.4 μM, 236.4 μM, and 56.9 μM, respectively (Figure S2 in the Supporting Information). Although **8i** showed some PPARɣ agonist activity, it is about 60 times more selective for GPR40.

To examine the effects of substituents on phenyl ring, the alkyl- (methyl, methoxyl, ethyl, or isopropyl) substituted derivatives did not show good GPR40 agonistic activity while the halogen substitution resulted in significant agonistic activity (Tables, 1 and 2). Chloro substitution (**8h - 8j**) provided good activity in the order *meta*- ≈ *ortho*- > *para*-position. At first, we examined if the meta substitution is important for activity, *meta*-bromophenyl analogue **8k** was synthesized, but its activity was about 50-fold lower than the *meta*-chloro derivative **8i**. Therefore, we focused on the ortho substitution assuming that the steric effect of *ortho* substituent may adjust the dihedral angle between the indole ring and the phenyl ring optimal for ligand binding (Table 2). Various *ortho*- substituted derivatives were prepared and their agonistic effects were evaluated. Among the alkyl substituted analogues, only *o*-methyl phenyl attached compound **8b**, showed submicromolar activity. Derivatives with *o*-CF_3 (**8l**), *o*-OCF_3 (**8m**), and *o*-F (**8n**) exhibited moderate micromolar potency. The compound having *o*-Br substituent (**8o**), showed the highest agonistic activity (EC_{50} = 9.4 nM) while the *m*-Br derivative showed almost 200 times lower activity (**Table 1**). These results suggest that a substituent at the *ortho* position of aryl group may not only be a simple steric blocker to adjust the conformation of the ligand, but may also be involved in interaction with the receptor. The halogen atom is preferred at the *ortho* position and the agonistic activity decreases in the order Br > Cl > F > H.

To understand the dramatic difference in GPR40 agonistic activity between *o*-Br (**8o**) and *m*-Br (**8k**) based on their binding modes, docking simulations were carried out using the X-ray structure of TAK875-GPR40 complex (pdb id = 4PHU).[23] Most GPR40 agonists have a carboxylic acid moiety as a key pharmacophore. The analysis of X-ray structure revealed a polar interaction network between the carboxylate group of TAK875 and Arg183, Arg258, Tyr91, and Tyr240, which is crucial for GPR40 agonistic activity. Likewise, in the docked models of GPR40 with **8o** and **8k** within the TAK875-binding pocket (Figure 3), the carboxylate moiety also forms hydrogen bonds with Arg183 and Arg258. In addition, indole N-H interacts with backbone amide carbonyl oxygen of Leu138 by forming a H-bond, suggesting that indole NH group may affect the receptor binding affinity. To verify the significance of indole NH for GPR40 agonistic effect, we prepared and tested several N-methylated indole derivatives of **8i**, **8j**, and **8o**, and found that they almost lost the activities with EC_{50} >10μM (**8s**, **8t**, and **8u** in the Supporting Information Table S1).

We also identified the unique Br•••π interaction[30, 31] between the Br substituent of drug and Phe142 in the binding site, as a possible key factor for GPR40 agonistic activity (Figure 3A). In the binding pose of **8o**, the distance from *o*-Br to the centroid of phenyl ring in Phe142 is 3.71Å and that to the nearest carbon of phenyl ring is 3.33Å, giving a difference of 0.38Å. Since this difference is bigger than 0.3Å, the Br•••π interaction can be formed with an "edge-on" geometry. However, for the *m*-Br analogue (**8k**), the distance from *m*-Br atom to the centroid of phenyl ring in Phe142 is 5.25Å (Figure 3B), and that to the nearest carbon of phenyl ring is 4.46Å. It means the Br•••π interaction cannot be formed, because both distances are longer than 4.2Å. Thus, the Br•••π interaction might contribute to provide higher binding affinity toward GPR40 for **8o**.

Table 2. Effect of ortho-substiuted aryl 2-indole propanoic acid



Compd	R	EC_{50} (nM, hFFA1)[a]	ALog P[b]	PPAR γ at 10μM
8l		1308	4.59	8%
8m		1016	5.78	2%
8n		1207	3.86	3%
8o		9.4	4.40	4%
8p		N.A.	4.59	7%
8q		N.A.	4.84	2%
8r		N.A.	2.78	64%

[a] Luciferase reporter assay, [b] AlogP [25] was predicted by schrödinger maestro, [c] % activity compared to 1μM rosiglitazone, N.A. no activity (> 25μM).

In most of the reported GPR40 agonists with a propanoic acid functional group, some substituents on its beta position were employed to improve the metabolic stability by avoiding β-oxidation. Therefore, we inspected a liver microsomal stability of compound **8h**, **8i** and **8o**, and they exhibited good metabolic stability (**Table 3**).

Table 3. Metabolic stability of compound **8h**, **8i**, and **8o**

Compound	$CL_{int.}$ (human / mouse)[a]
8h	9.2 / 14.3
8i	4.3 / 11.3
8o	7.1 / 10.4

[a] microsomal stability: Clearance intrinsic (μL/min/mg)

To detect insulin secretion effects of derivatives having GPR40 agonistic activity in the NFAT luciferase assay, we performed a glucose stimulated insulin secretion (GSIS) assay in MIN6 cells.[1] The insulin secretion effect was verified at a glucose concentration of 17mM. For compounds, **8h**, **8i** and **8o**, insulin secretion was elevated by 1.4-, 1.7-, and 1.6-fold compared to 17mM glucose, respectively (**Figure 4**). Compounds, **8i** and **8o** yielded GSIS effects similar to that of the reference compound TAK875 (1.7 fold). In contrast, the compound **8a** with low GPR40 agonist activity (EC_{50} > 25 μM)**,** did not induce insulin secretion. The result suggests that the GSIS effect of **8h**, **8i** and **8o** is related with selective GPR40 activation.

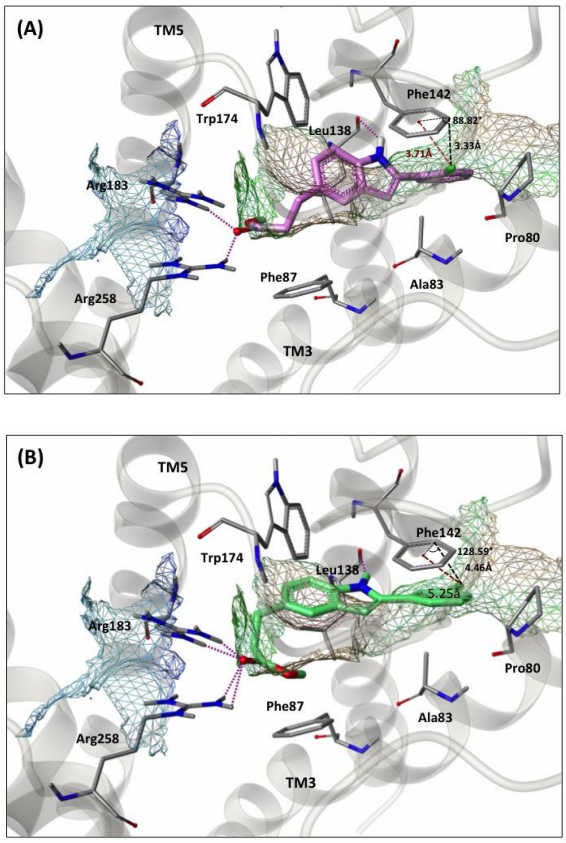


Figure 3. Computational analysis of the binding mode of 8o (A) and 8k (B) into GPR40. The carboxylate moiety is highly coordinated by several key res dues. Hydrogen bond interactions (<3Å) are depicted as dashed cylinders. Grey capped sticks represent key amino acid residues with in the binding site and ribbon is backbone of hGPR40. The binding site is rendered in MOLCAD lipophilic potential surface, and colored from blue (hydrophilic) to dark brown (hydrophobic).

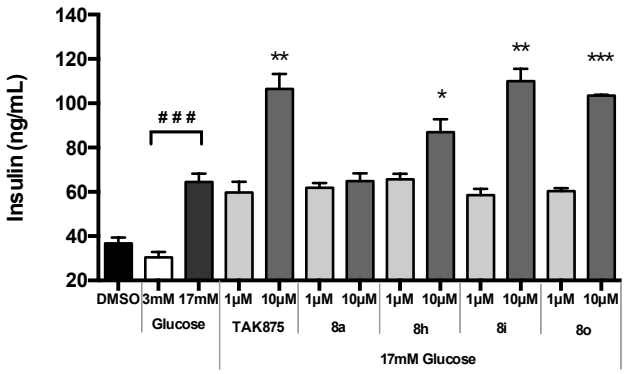


Figure 4. Glucose-stimulated insulin secretion test of derivative **8a**, **8h**, **8i**, and **8o** in MIN6 cells. The data represented as the mean ± standard error (n=2). ###p ≤ 0.01 by Student's test; *p ≤ 0.05, **p ≤ 0.025, ***p ≤ 0.01 vs 17mM glucose alone by Student's test.

In conclusion, for development of novel GPR40 agonists, we utilized indole propanoic acid moiety as a simplified novel scaffold for a potent GPR40 agonists. Various derivatives were prepared and their GPR40 agonistic activities were evaluated by an NFAT luciferase assay. Among these derivatives, potent GPR40 agonists were identified. These agonist, **8h**, **8i**, and **8o** showed good microsomal stability as well as an excellent insulin secretion effect in MIN6 cells. Based on these results, we continue to perform further *in vivo* efficacy study in due course for the development of more potent GPR40 agonists as anti-diabetic candidates.

Supporting Information

Syntheses and characterization data for the compound 8a-8u, assay protocols, and computational modeling

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Author Contributions

‖ Theses authors contributed equally.

Notes

The authors declare no competing financial interest.

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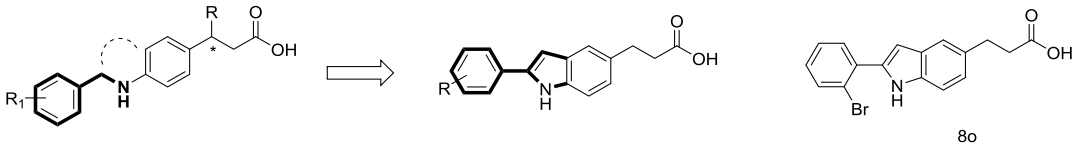

8o
EC_{50} = 9.4nM